Ribozymes & Anti inflammatory

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 NEWS  4  Feb 16     TOXLINE no longer being updated
 NEWS  5  Apr 23     Search Derwent WPINDEX by chemical structure
 NEWS  6  Apr 23     PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
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=> s ribozyme? (5w) inflammation
L1 4 RIBOZYME? (5W) INFLAMMATION

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2 4 DUP REM L1 (0 DUPLICATES REMOVED)
ANSWERS '1-2' FROM FILE CAPLUS
ANSWERS '3-4' FROM FILE BIOTECHDS

=> d ibib abs 1-4

L2 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:640975 CAPLUS
DOCUMENT NUMBER: 131:281543
TITLE: Ribozymes and methods for the treatment of diseases or conditions related to molecules involved in angiogenic responses
INVENTOR(S): Pavco, Pamela A.; Roberts, Elisabeth; Jarvis, Thale; Coeshott, Claire; McSwiggen, James A.
PATENT ASSIGNEE(S): Ribozyme Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 305 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9950403	A2	19991007	WO 1999-US6507	19990324
WO 9950403	A3	20010118		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
AU 9933647	A1	19991018	AU 1999-33647	19990324
EP 1086212	A2	20010328	EP 1999-915032	19990324

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

PRIORITY APPLN. INFO.:

US 1998-79678 P 19980327
WO 1999-US6507 W 19990324

AB Nucleic acid mols. which modulate the synthesis, expression, and/or stability of an mRNA encoding for angiogenic factors selected from aryl hydrocarbon nuclear transporter (ARNT, also known as HIF-1.beta.), integrin subunit .beta.3, integrin subunit .alpha.6, and Tie-2 (also known as Tek) are provided. The methods described herein represent a scheme by which ribozymes may be derived that cleave mRNA targets required for angiogenesis. The sequence of human mRNAs for the above angiogenic factors were screened for accessible sites using a computer folding algorithm. Regions of the mRNA that do not form secondary folding structures and contain potential hammerhead and/or hairpin ribozyme cleavage sites are identified. The sequences of 3324 such ribozymes and their target sites are provided. Also provided is a description of how such ribozymes may be delivered to cells. The examples demonstrate that upon delivery, the ribozymes inhibit cell proliferation in culture and modulate gene expression in vivo. Moreover, significantly reduced inhibition is obsd. if mutated ribozymes that are catalytically inactive are applied to the cells. Thus, inhibition requires the catalytic activity of the ribozyme. This invention further provides a treatment for indications related to angiogenesis using the nucleic acid mols., including but not limited to cancer, diabetic retinopathy, age-related macular degeneration, inflammation, and arthritis.

L2 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:464103 CAPLUS
DOCUMENT NUMBER: 131:84843
TITLE: Human I.kappa.B kinase .beta. subunit (IKK.beta.), its cDNA sequences, recombinant expression, and use in treating inflammation and in identifying anti-inflammatory drugs
INVENTOR(S): Chu, Keting; Pot, David
PATENT ASSIGNEE(S): Chiron Corp., USA
SOURCE: PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9934000	A1	19990708	WO 1998-US27917	19981230

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW

RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6030834	A	20000229	US 1998-215131	19981218
AU 9920242	A1	19990719	AU 1999-20242	19981230

PRIORITY APPLN. INFO.:

US 1997-68954 P 19971230
US 1998-215131 A2 19981218

WO 1998-US27917 W 19981230

AB The invention provides polynucleotides encoding the full length, N-terminal kinase domain and C-terminal HLH domain of human I.kappa.B kinase .beta. subunit (IKK.beta.). The cDNA sequence encoding these polynucleotides are provided. The invention also provides: (1) a fusion protein contg. a portion of IKK.beta.; (2) anti-IKK.beta. antibodies; (3) a probe able to hybridize to the IKK.beta. polynucleotides; (4) an expression construct contg. a promoter and a segment of the IKK.beta. polynucleotide; (5) and use of this construct to direct transcription of the IKK.beta. gene in a selected host cell. The invention further provides a method for identifying compds. that either inhibit the phosphorylation of I.kappa.B by IKK.beta. kinase or that inhibit binding of IKK.beta. kinase to I.kappa.B, which can be used as anti-inflammatory agents. Finally, the invention provides a method for treating inflammation involving administering a reagent, such as ribozyme, antibody, antisense oligonucleotide, which can inhibit the expression of the IKK.beta. gene kinase. The invention demonstrated that IKK.beta. can autophosphorylate and can also phosphorylate I.kappa.B.alpha..

REFERENCE COUNT: 6

REFERENCE(S):

(1) Maniatis, T; SCIENCE 1997, V278, P818 CAPLUS
(2) Mercurio, F; SCIENCE 1997, V278, P860 CAPLUS
(3) Nakano, H; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 1998, V95(7), P3537 CAPLUS
(4) Signal Pharm Inc; WO 9808955 A 1998 CAPLUS
(5) Univ California; WO 9837228 A 1998 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1999-08077 BIOTECHDS

TITLE: Nucleic acid encoding tumor necrosis factor receptor-like protein;
recombinant protein, antibody, DNA probe, DNA primer, antisense or triple helix oligonucleotide and **ribozyme** for use in cancer, **inflammation** or metabolic disease diagnosis or therapy

AUTHOR: Busfield S J

PATENT ASSIGNEE: Millennium-Biotherapeutics

LOCATION: Cambridge, MA, USA.

PATENT INFO: WO 9915663 1 Apr 1999

APPLICATION INFO: WO 1998-US20219 25 Sep 1998

PRIORITY INFO: US 1998-42785 17 Mar 1998; US 1997-938896 26 Sep 1997

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1999-254712 [21]

AN 1999-08077 BIOTECHDS

AB Nucleic acid encoding a tumor necrosis factor receptor-like protein (I) is claimed. (I) is at least 60% homologous with sequences of the 3,331 bp mouse, 2,612 bp human TRLI or 2,638 bp human TRLII cDNA with the insert of the plasmid deposited as ATCC 98544 (ATCC 98649 in disclosure) or their complements. (I) is a fragment with at least 500 nucleotides and encodes a protein (IIa) at least 60% homologous with 573, 253 or 605 amino acid mouse, human TRLI or human TRLII, respectively. Alternatively, (I) encodes a fragment of at least 15 amino acids from (IIa) and encodes a natural allele of (IIa). (I) hybridizes under stringent conditions with the 3,331 bp mouse, 2,612 bp human TRLI or 2,638 bp human TRLII cDNA. Also claimed are host cells containing (I); proteins of (II); (IIa) or fragments or alleles encoded by (I) or its

fragment or hybridizing sequences; antibodies specific for (II); recombinant (II) production; detection of (II) by reaction with a specific binding agent; detecting (I) by specific hybridization; and kits for the methods. Antisense and triple helix oligonucleotides, DNA primers, DNA probes, ribozymes and transgenic animals are disclosed. (170pp)

L2 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-02072 BIOTECHDS
TITLE: New human SMT3-like protein;
plasmid pINCY1 expression in Escherichia coli, DNA probe, antibody, agonist, antagonist, antisense and **ribozyme** for cancer and **inflammation** therapy
AUTHOR: Hillman J L; Shah P
PATENT ASSIGNEE: Incyte-Pharm.
LOCATION: Palo Alto, CA, USA.
PATENT INFO: WO 9850545 12 Nov 1998
APPLICATION INFO: WO 1998-US8420 6 May 1998
PRIORITY INFO: US 1997-853974 9 May 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1999-034720 [03]
AN 1999-02072 BIOTECHDS
AB A substantially purified human SMT3-like protein (126 amino acids) is new. Also claimed are: a polynucleotide which hybridizes or is complementary to the SMT3-like nucleotide; an expression vector; a host cell; SMT3-like protein antibodies, agonists and antagonists; antisense molecules/ribozymes; and pharmaceutical compositions containing the above. The nucleic acid encoding the protein was identified in a fetal lung cDNA library. Expression of the protein is associated with fetal development, inflammation, cancer and radiation damage. It is used to promote DNA repair/treat patients with ataxia telangiectasia or related diseases who are undergoing radiation treatment for cancers associated with these diseases and can be added to sunscreens. In an example, the cDNA sequence for SMT3-like protein was cloned into plasmid pINCY1, the product used to transform Escherichia coli and cells induced with IPTG to produce a fusion protein. This comprised the first 8 residues of beta-galactosidase (EC-3.2.1.23), 5-15 linker residues and the full-length sequence and was secreted into the culture medium from a signal peptide. (54pp)

L4 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:261342 CAPLUS

DOCUMENT NUMBER: 120:261342

TITLE: Treatment of inflammatory diseases with ribozymes

INVENTOR(S): Sullivan, Sean M.; Draper, Kenneth G.

PATENT ASSIGNEE(S): Ribozyme Pharmaceuticals, Inc., USA

SOURCE: PCT Int. Appl., 65 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 31

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9402595	A1	19940203	WO 1993-US6316	19930702
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 654077	A1	19950524	EP 1993-918144	19930702
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 07509133	T2	19951012	JP 1993-504490	19930702
EP 786522	A2	19970730	EP 1997-101534	19930702
EP 786522	A3	19970827		
R: AT, CH, DE, ES, FR, GB, IT, LI, SE				
US 5817796	A	19981006	US 1995-435628	19950505
AU 729657	B2	20010208	AU 1998-51819	19980112
AU 9851819	A1	19980611		
AU 9852096	A1	19980319	AU 1998-52096	19980116
AU 9939188	A1	19990916	AU 1999-39188	19990713

PRIORITY APPLN. INFO.:

US 1992-916763	A	19920717
US 1992-987132	A	19921207
US 1992-989848	A	19921207
US 1992-989849	A	19921207
US 1993-8895	A	19930119
US 1992-936422	B1	19920826
EP 1993-918144	A3	19930702
WO 1993-US6316	W	19930702
US 1994-192943	A2	19940207
US 1994-245466	B2	19940518
US 1995-373124	A3	19950113
AU 1995-26422	A3	19950518
US 1996-623891	A	19960325

AB Enzymic RNA mols. that cleave mRNAs assocd. with development or maintenance of an inflammatory disease, an arthritic condition, a stenotic condition, or a cardiovascular condition are described for use in the treatment of disease. Possible targets include mRNAs for tumor necrosis factor, interleukins, adhesion mols., selectins, proteinases, kinases, and growth factors. Possible cleavage sites for hammerhead ribozymes on a no. of mRNAs are identified; the ribozymes may be synthesized chem. or by expression of the gene. Stability of ribozymes in cytoplasmic exts. of cultured animal cells was tested; it was found that a divalent cation-dependent nuclease was involved in the degrdn. of the ribozyme. Methods for delivery of the ribozyme are described.

L4 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:824045 CAPLUS

DOCUMENT NUMBER: 133:359232

TITLE: Anti-inflammatory therapy for inflammatory-mediated infection

INVENTOR(S): Anton, Peter A.; Poles, Michael A.; Giorgi, Janis V.; Elliott, Julie E.

PATENT ASSIGNEE(S): The Regents of the University of California, USA

SOURCE: PCT Int. Appl., 97 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000069255	A1	20001123	WO 2000-US13142	20000512

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-134091 P 19990514

AB Methods are provided for inhibiting the progression of an inflammatory-mediated mucosal infection. The methods include administering an effective amt. of an anti-inflammatory agent. Also provided are compns. and articles of manuf. for preventing, and inhibiting the activation and progression of a mucosal infection.

REFERENCE COUNT: 9

REFERENCE(S):

(1) Aggarwal; US 5891924 A 1999 CAPLUS
(4) Bernton; US 5605885 A 1997 CAPLUS
(5) Bourinbaiar; Biochemical and Biophysical Research Communications 1995, V208(2), P779 CAPLUS
(6) Goletti; Journal of Infectious Diseases 1998, V177(5), P1332 CAPLUS
(8) Ornstein; Arthritis and Rheumatism 1996, V39(1), P157 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:231448 CAPLUS
DOCUMENT NUMBER: 126:288105
TITLE: Ribozymes cleaving interleukin-5 mRNA for treatment and diagnosis of asthma and other inflammatory disorders
INVENTOR(S): Sullivan, Sean; Draper, Kenneth G.; McSwiggen, James; Stinchcomb, Dan T.
PATENT ASSIGNEE(S): Ribozyme Pharmaceuticals, Inc., USA
SOURCE: U.S., 145 pp. Cont.-in-part of U.S. Ser. No. 989,849, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 31
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5616488	A	19970401	US 1994-319492	19941007
EP 786522	A2	19970730	EP 1997-101534	19930702
EP 786522	A3	19970827		
R: AT, CH, DE, ES, FR, GB, IT, LI, SE				
CA 2183992	AA	19950831	CA 1995-2183992	19950223
WO 9523225	A2	19950831	WO 1995-IB156	19950223
WO 9523225	A3	19960201		
W: AU, CA, JP, KR, MX				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 746614	A1	19961211	EP 1995-909920	19950223
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 09509323	T2	19970922	JP 1995-522236	19950223
US 5616490	A	19970401	US 1995-434503	19950504
AU 729657	B2	20010208	AU 1998-51819	19980112
AU 9851819	A1	19980611		
AU 9939188	A1	19990916	AU 1999-39188	19990713
AU 9947567	A1	19991104	AU 1999-47567	19990913

PRIORITY APPLN. INFO.:

US 1992-989849	B2	19921207
US 1993-8895	B2	19930119
US 1992-916763	A	19920717
US 1992-987132	A	19921207
US 1992-989848	A	19921207
EP 1993-918144	A3	19930702
US 1994-201109	A	19940223
US 1994-218934	A	19940329
US 1994-222795	A	19940404
US 1994-224483	A	19940407
US 1994-227958	A	19940415
US 1994-228041	A	19940415
US 1994-245736	A	19940518
US 1994-271280	A	19940706
US 1994-291932	A	19940815
US 1994-291433	A	19940816
US 1994-292620	A	19940817
US 1994-293520	A	19940819
US 1994-300000	A	19940902
US 1994-303039	A	19940908
US 1994-311486	A	19940923

US	1994-311749	A	19940923
US	1994-314397	A	19940928
US	1994-316771	A	19941003
US	1994-319492	A	19941007
US	1994-321993	A	19941011
US	1994-334847		19941104
US	1994-337608		19941110
US	1994-345516		19941128
US	1994-357577		19941216
US	1994-363233		19941223
US	1995-380734		19950130
WO	1995-IB156	W	19950223
AU	1995-26422	A3	19950518
US	1995-475460	A	19950607
US	1995-483715	A	19950607
US	1995-484607	A	19950607
US	1996-623891	A	19960325
AU	1996-61744	A3	19960603

AB Ribozymes that cleave the mRNA of interleukin 5 are described for use in the therapeutic control of interleukin levels in the treatment of asthma and other inflammatory diseases. Interleukin 5 levels are shown to be raised in bronchoalveolar lavage and lung biopsies of asthma patients, implying a role for helper T-cells in the inflammatory response.

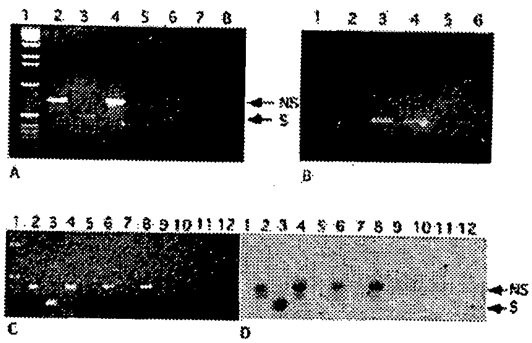

Figure 2. Transcription and splicing of mouse genes by *S.cerevisiae*. **(A)** *Dll1* locus (Table 1, exp. 2). Lane 1, DNA marker; lane 2, FFEH11 total yeast DNA PCR (genomic DNA control); lane 3, mouse cDNA PCR (spliced control); lane 4, FFEH11 random primed RT–PCR; lane 5, RT– control of lane 4; lane 6, the same as in lane 4, but RNA was degraded with RNase A prior RT (abbreviated hereupon RN–); lane 7, unrelated YAC clone RT–PCR (henceforth YAC–); lane 8, water control. The product in lane 4 was sequenced and found to correspond to the unspliced *Dll1* gene. S, spliced; NS, not spliced product. **(B)** Splicing of the mouse *Psmb1* gene by the yeast (exp. 8), stained agarose gel. Lane 1, DNA marker; lane 2, genomic DNA control; lane 3, mouse cDNA spliced control; lane 4, FFEH11 random primed RT–PCR; lane 5, RT– control; lane 6, RN– control. The product in lane 4 was sequenced and found to correspond to the correctly spliced transcript of the *Psmb1* gene. **(C)** *D17Ph4e* locus is transcribed from both DNA strands (exp. 7). Lane 1, DNA marker; lane 2, genomic DNA control; lane 3, mouse cDNA spliced control; lane 4, FFEH11 oligo(dT) primed RT–PCR; lane 5, RT– control; lane 6, FFEH11 RT primed with a sense oligo (77B); lane 7, RT– control for lane 6; lane 8, FFEH11 RT primed with an antisense oligo (5F); lane 9, RT– control for lane 8; lane 10, RN– control; lane 11, YAC– control; lane 12, water control. The product in lane 4 was sequenced and found to correspond to the unspliced part of the *D17Ph4e* gene. **(D)** Autoradiograph of Southern blot of the agarose gel from (C) hybridized with a *D17Ph4e* probe.

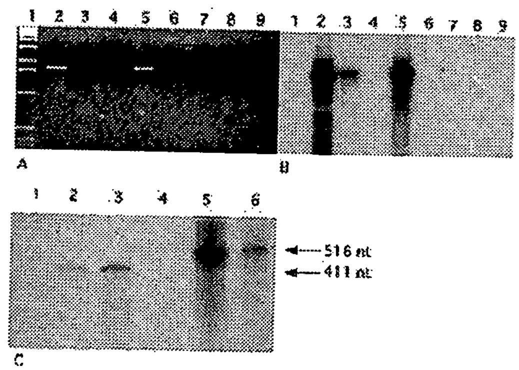

Figure 3. Promiscuous transcription of mouse non-coding DNA in the yeast (Table 1, exp. 14). RT–PCR detects an RNA transcript from the intergenic region between the first exons of the *Tbp* and *Psmb1* genes: lane 1, DNA marker; lane 2, genomic DNA control; lane 3, FFEH11 random primed RT–PCR; lane 4, RT– control; lane 5, FFEH11 oligo(dT) primed RT–PCR; lane 6, RT– control for lane 5; lane 7, RN– control; lane 8, YAC– control; lane 9, water control. **(A)** stained agarose gel; **(B)** autoradiograph of the blotted gel hybridized with a sequenced probe from the *Psmb1* promoter region. **(C)** RNase protection assay with an RNA probe for the intergenic region between the *Tbp* and *Psmb1* genes. Lane 1, yeast RNA control (10 µg); lane 2, FFEH11 RNA (10 µg); lane 3, FFEH11 RNA (35 µg); lane 4, mouse RNA (2 µg); lane 5, no RNase; lane 6, elution control. The arrows mark the sizes of the eluted (516 bp) and fully protected (411 bp) RNA probe.

the YAC RT–PCR sequence spans only 58 bp of the 3′ intergenic region of the *Psmb1* gene, and that the rodent *Psmb1* gene transcript carries two alternative polyA signals. The second mouse positioning signal (AATAAA) is surrounded by three hexanucleotides (two of them overlapping) similar to the yeast efficiency polyA signal (TATDTA). While both the yeast and mouse polyA signals are putative, detected by computer searching for consensus sequences downstream to polyA sites, the polyA sites were detected experimentally by cDNA sequencing. Neither mouse polyA signal is used by the yeast cell, as the 3′ RACE sequence exceeds the length of any mouse *Psmb1* mRNA, and the putative yeast polyadenylation site is located downstream to two overlapping TATDTA yeast signals.

DISCUSSION

Previous studies on yeast and mammalian gene expression revealed common features as well as differences in their transcription, splicing and polyadenylation mechanisms. It has been documented that yeast can recognize mammalian promoters (13–15), but yeast and mammals vary in their requirements for the sequence context of the TATA boxes (16). Also, the intron splicing signals show several differences in spite of the common GT–AG rule, shared across the eukaryotic kingdom. For example, the yeast 3′ splicing signal consensus does not have a polypyrimidine stretch preceding the AG sequence, and the branching site is more conserved in the yeast than in mammals (17,18).

The mammalian polyadenylation signals are not generally recognized in *S.cerevisiae* (19,20). Although the yeast and mammalian signals are both composed of at least three elements, the yeast efficiency elements are different and the other two elements are more degenerate than the mammalian signals (reviewed in 21). The yeast efficiency signal optimal sequence is TATATA and hexanucleotides TATDTA have some activity, but several non-optimal elements are usually located downstream to the polyA site (22).

In spite of all these differences, Still *et al.* (4) reported successful screening for human genes in yeast RNA from the yeast clones carrying overlapping human YACs. Of 27 differentially expressed RNA fragments tested, the expression of four clones in human tissues (15%) was proven by PCR screening of five human cDNA libraries or by their match to expressed sequence tags. The specificity of the processing of the human genes by the yeast was not tested.

In the present report, we took advantage of a well characterized mouse YAC clone (7,10,11) to determine how efficient and specific is the yeast processing of the mouse DNA. Transcripts from five tested mouse genes encoded within the YAC clone were all found in the total yeast RNA. Of 12 mouse introns assayed, six were correctly spliced by the yeast. Besides the transcripts of exon sequences, 'yeast-specific' transcription of the YAC DNA was observed. At least three genes were transcribed from their sense and antisense strands. Microsatellite, inter-repetitive, and anonymous mouse loci were detected in random- and oligo(dT)-primed YAC RNA. A pair of primers derived from the first exons of two head-to-head oriented mouse genes yielded an RT–PCR product. An RNA probe, derived from this intergenic region, was wholly

protected by the YAC RNA in an RNase protection assay. This finding indicates that the steady state levels of transcribed RNA from the non-coding mouse sequences are high enough to be detected by a technique other than RT–PCR. The sequence analysis of a 3′ RACE product has shown that, in agreement with expectation (19,20), the mouse polyadenylation signals are not used by the yeast cell.

How to explain the observed high frequency of illegitimate transcription of mouse DNA in the yeast? The presence of unspliced introns, transcripts from both DNA strands or from microsatellite repeats could be easily understood, if genomic DNA rather than reverse transcribed cDNA acted as a template for PCR amplification. However, we have effectively ruled out this possibility not only by including the RNase-free DNase treatment and RT minus controls, but also by abrogation of the PCR signal by RNA treatment with DNase-free RNase before reverse transcription. Moreover, when the primers for intergenic endogenous yeast region were used, the RT–PCR did not yield any signal. No products were obtained also when two yeast genes were amplified from sense-oligo primed RT reactions.

Yeast transcription initiation complex recognizes the TATA box consensus, but it is not sensitive to the TATA box sequence context that is preferred by the mammalian transcription complex (16). Thus, due to a high ratio of non-coding to coding DNA in mammals, some random mouse sequences could serve as promoters in the yeast and explain the presence of transcripts from YAC non-coding regions or a non-template DNA strand. The same argument may hold true for TATA-less transcription initiation.

The splicing of six mouse introns (out of 12 tested) described in this report provides the first piece of evidence for a successful splicing of mammalian introns by yeast. The failure to splice all introns could be explained by the fact that the branching site sequence is more conserved in the yeast compared with mammals, and its variation can abolish splicing in the yeast (18). Admittedly, splicing signals of four analyzed introns did not show any apparent differences that could distinguish three non-spliced introns from one that was spliced. None of the examined mouse introns displayed a branching site identical to the yeast consensus. One possible explanation could be that these introns are spliced only partially and the non-spliced product was not detectable by our method. It may be of some interest that though the yeast introns are generally short, at least one of the successfully spliced mouse introns spanned >5 kb. Our study has suggested that although mammalian polyA signals are not used by yeast, the YAC RNAs can be polyadenylated, apparently due to the redundant occurrence of the degenerate yeast polyA signal sequences.

The present report shows that the yeast transcription apparatus transcribes mouse coding and non-coding sequences with comparable efficiency. The enrichment with mammalian mRNA in YAC RNA seems rather low and a method based on a selection of mammalian mRNA in the YAC clone RNA would be expected to produce a high background. For example, a hypothetical mouse gene of 25 and a mRNA of 2 kb in size would be transcribed and spliced by the yeast into an RNA >12 kb, provided that half of its introns were properly spliced. The enrichment would be even lower if the gene was transcribed from a random sequence recognized by the yeast transcription apparatus on a non-template DNA strand that cannot be spliced at all. The YAC clone under study covers a GC-rich region, as judged by the frequency of rare-cutter restriction sites (10). Such regions are gene-rich and under-represented in mammals (23). Although it cannot be *a priori* excluded that the YAC transcription and RNA processing would be more faithful in GC-poor YAC clones, the expected occurrence of TATA and TATDTA sequences, and thereby of possible transcription initiation and RNA 3′ end formation sites is higher.

The results presented here indicate that YAC clones can serve as *in vivo* test tubes for further analysis of the conservation of gene processing sequences. An intriguing possibility emerges to develop new yeast host strains capable of recognizing some mammalian DNA and/or RNA processing signals, and thus enriching RNA of YAC clones with mammalian exons.

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